The Role of Monovalent Cations in the Catalytic Mechanism of Fructose-1,6-bisphosphatase.

J. Choe, C. Iancu and R. Honzatko (Iowa State U.)

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Introduction: Fructose-1,6-bisphosphatase (FBPase) is a key regulatory enzyme in gluconeogenesis. The inhibition of FBPase in mammals results in reduced levels of serum glucose in the fasting state. Hence FBPase is a target for the development of drugs for the purpose of treating noninsulin dependent diabetes (diabetes type II). The catalytic rate of FBPase is controlled by an allosteric inhibitor (AMP), a competitive inhibitor (fructose-2,6-bisphosphate), divalent and monovalent metal cations, and the conformational state of the enzyme (R or T). The kinetic mechanism of FBPase depends on the type of metal cation at its active site.

Methods and Materials: Crystals of both R- and T-state complexes grew from hanging drops with 10 mg/ml of protein, 100mM Hepes, 5mM F6P, 10mM Kpi, PEG 3350 and various metal cations at different concentrations. Typical crystal measured 0.2mm $\times$ 0.2mm $\times$ 0.2mm. Data collected from flash-cooled crystals, using the beam line at X12C, were reduced and scaled by the HKL package. Crystals belong to space group I 2 2 2 (R-state) or P 21 21 21 (T-state). Initial phases were calculated from PDB code 1cnq, 1eyi, 1eyj or 1eyk. The refinement and the calculation of SAD anomalous difference maps were done using CNS.

Results: Anomalous difference maps reveal 3 monovalent cation sites for Tl^+ or Rb^+ in the presence of 2 bound Mg^{2+} in the two principal conformations of FBPase (R- and T-states). Concentrations of monovalent cations are approximately 20 mM, near reported Km values for these ions. Hence, all three monovalent cation sites and the two divalent cation sites may influence catalysis. Monovalent metals associate with catalytically essential residues. The monovalent cations may stabilize negative charge developed during the transition state. The use of the anomolous signal from Tl^+, using radiation of λ=0.979 A, allowed an unambiguous determination of the extent to which the monovalent cations replaced Mg^{2+} at the divalent cation sites.

In addition to the above, we obtained several structures of mutant forms of FBPase in complexes with divalent cations and products, in support of efforts to determine the allosteric mechanism of inhibition of FBPase by AMP.